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sectional area (specific force). This indicates defective excitation-contraction coupling. In this process, sarcoplasmic reticulum (SR) Ca^{2+} release via the ryanodine receptor 1 (RyR1) is a pivotal step that grades muscle contractile force. It has previously been shown that impaired contractility and SR Ca^{2+} release in muscular dystrophy can be caused by excessive RyR1-cysteine nitrosylation and reduced binding of the stabilizing protein FK506 binding protein 12 (FKBP12 or calstabin1) to RyR1. We hypothesized that maladaptations in the RyR1-SR Ca^{2+} release system could underlie impaired muscle function also in aging. Using immunoprecipitation and immuonblotting, we found that RyR1 from aged (24-26 month) mouse muscle were oxidized, cysteine-nitrosylated, and depleted of FKBP12, compared to RyR1 from younger (3-6 months) adult mice. This remodeling of the RyR1 resulted in ''leaky'' channels, which displayed an increased open probability and Ca^{2+} spark frequency.Moreover, tetanic Ca^{2+} transients and muscle specific forcewere reduced in 24-month-old mice. Treating aged mice with the RyR-stabilizing compound, S107, restored RyR1-FKBP12 interaction, and improved tetanic Ca^{2+} release, muscle specific force and exercise capacity.-Together, these findings highlight the role of impaired SR Ca^{2+} release in age-dependent muscle weakness and introduce a novel therapeutic target for sarcopenia.

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Pge$_2$ Accelerates Myogenesis of C2c12 Myoblasts

ChengLin Mo, Sandra Romero-Suarez, **Marco A. Brotto**.

Prostaglandin E2 (PGE_2), a modulator of physiological responses including inflammation, fever, and smooth muscle tone, is a prostanoid synthesized from arachidonic acid via the cyclooxygenase pathway.PGE_2 is released in large amounts by injured muscle fibers, and is linked to muscle regeneration by regulating myoblast fusion. However, the physiological roles and related molecular mechanisms of PGE_2 on skeletal muscle function remain elusive. We used C2C12 cells, to investigate the functional, cellular, and molecular effects of PGE_2. Treatment of C2C12 myoblasts to 50nM PGE_2 resulted in accelerated myogenesis when compared to the naturally potent effects of differentiating media containing 2.5% horse serum. To investigate the genetic mechanisms underlying these effects, we used a custom-built 96 real-time gene PCR array to search for genes and pathways that might help explain the myogenic effects of PGE_2. We discovered that PGE_2 robustly upregulates the MURF-NFKB calcium-dependent myogenic pathway in C2C12 myoblasts. We are now studying the functional and cell biological consequences of PGE_2 effects and its roles in muscle myogenesis and differentiation.

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PinhãO-Manso: A Native Jatropha Curcas from Brazil Protects Muscle Cells from Ethanol Toxicity and Improves Muscle Function

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Jatropha curcas is a perennial plant of the spurge family (Euphorbiaceae) typical of semi-arid regions in the world spanning from Brazil to China. Recently, it has received much attention as a potential source of vegetable oil as a replacement for petroleum, and, in particular, the production of biodiesel. An understudy aspect of this plant relates to its potential utilization as a pharmacological agent. Interestingly, the native plant (Pinhão-Manso, PM) of the semi-arid region of Brazil has been used as an ant-fatigue agent and also for healing of abrasions, cuts, and wounds.We used C2C12 muscle cells in the myoblast stage to begin our investigation of the functional, cellular, and molecular effects of PM. Overnight exposure of C2C12 myoblasts to 3% ethanol (vehicle) induced 100% cell death. We document here a fascinating result: when C2C12 were exposed to the same concentration of ethanol but in the presence of PM, cell death was prevented. Furthermore, in ex-vivo, intact isolated muscles from mice (extensor digitorum longus and soleus muscles); PM increased contractile force production and also prevented the toxic effects of alcohol on muscle function. We are now focusing on purification of active principle(s) of PM that might underlie its potent and beneficial effects on muscle function. We are also studying the molecular machinery that is modulated by PM. Our studies indicate that native Jatropha curcas from Salvador, Brazil could have potential applications for improvement of skeletal muscle function.

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Transcriptional Repression of Caveolin-1 gene Expression by Gata-6 in Bladder Smooth Muscle Hypertrophy in Mice and Humans

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Caveolin-1 is a structural and functional protein of caveolae that function as signaling platforms to mediate interaction between receptor proteins and adaptor and effector molecules to regulate signal generation, amplification, and diversification. Hypertrophied bladder smooth muscle (BSM) from the rabbit model of partial bladder outlet obstruction (PBOO) and men with benign prostatic hyperplasia (BPH)-induced PBOO shows a downregulation of Caveolin-1 and caveolar structural alteration. Here we report that caveolin-1 expression is diminished in PBOO-induced BSM hypertrophy in mice and in men with BPH. We characterized the proximal promoterof the human and mouse caveolin-1 gene and found that the transcriptionfactor GATA-6 binds this promoter, causing reduced expression of caveolin-1. Furthermore, we demonstrate that caveolin-1 expression levels inversely correlate with the abundance of GATA-6 in BSM hypertrophy in mice and humans. Importantly, silencing of GATA-6 gene expression up-regulates caveolin-1 expression, whereas overexpression of GATA-6 protein sustains the transcriptional repression of caveolin-1 in BSM cells. Together, our data suggest that GATA-6 acts as a transcriptional repressor of caveolin-1 gene expression in PBOO-induced BSM hypertrophy in men and mice. The GATA-6-induced transcriptional repression represents a new regulatory mechanism for caveolin-1 gene expression in pathologic BSM. Regulating the GATA-6 transcriptional regulation may serve as a target for new therapy for BPH-induced bladder dysfunction in aging men. Supported by O'Brien Urology Center Grant P50 DK052620

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Between a Rock and a Hard Place: Mitochondria Deform Anisotropically in Intact Cardiomyocytes During Active Contraction

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The cardiomyocyte cytoskeleton, composed of rigid and elastic elements, maintains the shape of an elongated cylinder with an eccentrically-ellipsoidal cross-section, even during contraction-relaxation cycles. Mitochondria are micron-sized fluid-filled passive spheres distributed throughout the cardiomyocyte in a crystal-like lattice in pairs sandwiched between the sarcomere contractile machinery, longitudinally and radially; thus, their shape represents the balance of forces in 3D extant at any given moment. We developed a novel method to examine the average deformation of mitochondrial dimensions in 3D, in response to cardiomyocyte contraction and relaxation, to understand how dynamic forces are balanced inside the cardiomyocytes. The optical contrast provided by the periodic lattice of myofilaments alternating with rows of mitochondria was analyzed by examining the appropriate peaks in the frequency spectrum image along the respective cardiomyocyte axes. This technique enables precise resolution of changes in dimension of ~1% in ~1 μm (long axis) structures with a time resolution of 8 msec.

During active contraction (1 Hz stimulation) the mitochondria deform along the length-and width-axes with similar time-to-peak deformation and 50% and 90% deformation duration characteristics in both sarcomere and mitochondrial structures. However, significant deformation anisotropy was observed between the orthogonal short (i.e., width & depth) axes of *mitochondria* during electrical stimulation. Interestingly, the same degree of deformation anisotropy was found between the *myocyte* orthogonal short axes during the same electrical stimulation; therefore, the mitochondria reflect the overall cell behavior, and the apparent stiffness and stress/strain characteristics of the cytoskeleton differ appreciably between the cardiomyocyte orthogonal short axes. This method may be applied to obtaining a better understanding of the dynamic force-balance inside cardiomyocytes and of changes in the cytoskeleton spatial stiffness characteristics that may accompany aging or pathological conditions.